

# Significant association of *BDNF* rs6265 G>A polymorphism with susceptibility to epilepsy: a meta-analysis

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**Introduction:** Previously published articles have suggested that *BDNF* rs6265 G>A polymorphism is a potential risk factor for epilepsy. However, the results were not consistent.

**Methods:** We conducted a meta-analysis to explore the association between *BDNF* rs6265 G>A polymorphism and epilepsy risk. Four online databases were searched, and related studies were reviewed from their inception up to June 20, 2017. ORs and corresponding 95% CIs were used to calculate the associations of each genetic model. Overall, 10 case-control publications involving 9,512 subjects were included in this meta-analysis.

**Results:** Significant associations were found between *BDNF* rs6265 G>A polymorphism and epilepsy (A vs G: OR=0.88, 95% CI=0.83–0.94,  $P<0.01$ ,  $I^2=0\%$ ; GA vs GG: OR=0.88, 95% CI=0.79–0.97,  $P=0.01$ ,  $I^2=0\%$ ; AA vs GG: OR=0.79, 95% CI=0.70–0.90,  $P<0.01$ ,  $I^2=0\%$ ; GA+AA vs GG: OR=0.85, 95% CI=0.77–0.94,  $P<0.01$ ,  $I^2=0\%$ ; AA vs GG+GA: OR=0.85, 95% CI=0.76–0.95,  $P=0.01$ ,  $I^2=0\%$ ). Subgroup analysis also showed similar results in an Asian population.

**Conclusion:** Our meta-analysis indicated that *BDNF* rs6265 G>A polymorphism might be involved in epilepsy susceptibility, especially in the Asian population.

**Keywords:** brain derived neurotrophic factor, epilepsy, seizure, polymorphism, variant, rs6265, case-control

## Introduction

Epilepsy is a complex neurological disease that involves transient disturbances in cerebral function, which is mostly caused by pathologic neuronal discharges and characterized by recurrent and spontaneous seizures. To date, more than 50 million people worldwide have epilepsy.<sup>1</sup> The prevalence of epilepsy is approximately 0.5%–1%,<sup>2</sup> with a total annual incidence of 50–70 or 190 cases per 100,000 individuals in industrialized and developing countries.<sup>3</sup> At least 80% of epilepsy patients live in a developing country,<sup>4,5</sup> and only 25% of patients receive basic treatment.<sup>6</sup> In 2017, the International League against Epilepsy (ILAE) published a revised operational classification of seizure types with focal onset, generalized onset, and unknown onset.<sup>7</sup>

Morbidity due to epilepsy is more common in children and the elderly.<sup>8,9</sup> The brain has been proven to be more “seizure susceptible” in childhood and old age. The immature brain mechanisms and degenerative changes may lead to an increased risk of epileptic seizures.<sup>10,11</sup> Epilepsy is a serious threat to health and will lead to a significant economic burden for the patients and their families, as well as decreased quality of life. Today, epilepsy is not only a disease but also a serious problem in terms of public health, social stability, and economic development. However, current treatments focus on managing

symptoms with a lot of antiepileptic drugs because the pathogenesis of epilepsy remains unclear. In addition, the treatment and prognosis for epilepsy are not yet satisfactory.

In the past decade, *BDNF* has been recognized as one of the important neurotrophic factors that could regulate the morphology of neurons and synapse formation, playing a neuroprotective role in the central nervous system.<sup>12</sup> Animal model studies with transgenic mice also demonstrated that overexpression of *BDNF* in the brain can interfere with increased excitability and epilepsy risk.<sup>13,14</sup>

Single-nucleotide polymorphisms (SNPs) are drawing increased attention in current studies, because they are one of the most important genetic mutations and could regulate *BDNF* expression and metabolism. A large amount of research has proven that rs6265 G>A (with a nucleotide change from guanine deoxyribonucleotide to adenine deoxyribonucleotide) is the most common polymorphism located in the *BDNF* gene that causes an amino acid change from valine (Val) to methionine (Met), which may contribute to various central nervous system diseases, such as Alzheimer's disease,<sup>15</sup> Parkinson's disease,<sup>16</sup> depression,<sup>17</sup> and bipolar disorder.<sup>18</sup> In 2003, Kanemoto et al published the first study on the association between *BDNF* rs6265 G>A polymorphism and epilepsy susceptibility and did not find any significant association in a Japanese population.<sup>19</sup> To date, subsequent studies have been published about the relationship between *BDNF* rs6265 G>A polymorphism and epilepsy susceptibility, but the results were controversial. We therefore conducted this meta-analysis to more precisely assess the association between *BDNF* rs6265 G>A polymorphism and epilepsy susceptibility.

## Methods

This meta-analysis was conducted under the guidance of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.<sup>20</sup> All included data were collected from published studies, and there were no ethical issues.

## Search strategy

Four online databases (PubMed, Embase, CNKI, and Wanfang) were searched for relevant studies with the following search strategy on the association between *BDNF* rs6265 G>A polymorphism and epilepsy risk from their inception up to June 20, 2017. The bibliographies of the collected studies and relevant reviews were retrospectively assessed to identify additional articles. Only studies that were written in English

and Chinese were included. The following search terms and strategy were used (for example in PubMed):

#1 *Brain Derived Neurotrophic Factor*

#2 *BDNF*

#3 rs6265

#4 #1 OR #2 OR #3

#5 polymorphism

#6 variant

#7 mutation

#8 #5 OR #6 OR #7

#9 epilepsy

#10 seizures

#11 #9 OR #10

#12 #4 AND #8 AND #11.

## Study selection and exclusion

All selected studies shared the following inclusion criteria: 1) case-control and cohort studies; 2) studies focused on *BDNF* rs6265 G>A polymorphism and epilepsy risk; 3) all data were based on human subjects; and 4) selected with adequate genotype information or completed OR and 95% CIs. The exclusion criteria included: 1) case report or review articles; 2) molecular biology research; 3) articles without enough data or that could not be calculated to corresponding OR and a 95% CI; and 4) articles with duplicated or overlapping data.

## Data extraction

Two reviewers (Xu and Li) retrieved, read, and extracted the relevant data from selected studies independently. This information was collected and listed in Table 1: author, study publication year, country or ethnic distribution of the subjects, control design, genotyping method, subtype of epilepsy, the distributed number of genotypes in cases and controls, the group and patient age, and the minor allele frequency in the control group.

## Statistical analysis

The Hardy-Weinberg equilibrium (HWE) in controls of each study was calculated by using a chi-square test first. Pooled ORs with 95% CIs were calculated to evaluate the strength of the relationship between *BDNF* rs6265 G>A polymorphism and epilepsy risk. Five genotype models were used, including allele contrast (A vs G), co-dominant (GA vs GG and AA vs GG), dominant (GA+AA vs GG), and recessive (AA vs GG+GA) models. Subgroup analyses were also conducted according to stratified design, such as ethnic distribution, control design, disease type, age distribution, and genotyping method. Cochran's Q statistic was used to

**Table 1** Characteristics of case-control studies on BDNF rs6265 G>A polymorphism and epilepsy risk included in the meta-analysis

| First author                 | Year | Country/ethnicity    | Control design   | Genotyping method  | Case  | Control | Genotype distribution |     |     |         |       |       | P-value for HWE <sup>a</sup> | Type      | AD    | MAF  |
|------------------------------|------|----------------------|------------------|--------------------|-------|---------|-----------------------|-----|-----|---------|-------|-------|------------------------------|-----------|-------|------|
|                              |      |                      |                  |                    |       |         | Case                  |     |     | Control |       |       |                              |           |       |      |
|                              |      |                      |                  |                    |       |         | GG                    | GA  | AA  | GG      | GA    | AA    |                              |           |       |      |
| Kanemoto et al <sup>19</sup> | 2003 | Japan/Asian          | Hospital control | PCR-RFLP           | 219   | 325     | 66                    | 116 | 37  | 105     | 164   | 56    | 0.56                         | TLE+other | Adult | 0.42 |
| Chou et al <sup>28</sup>     | 2004 | China/Asian          | Healthy control  | PCR-RFLP           | 103   | 83      | 29                    | 46  | 28  | 16      | 38    | 29    | 0.29                         | Epilepsy  | Child | 0.58 |
| Lohoff et al <sup>21</sup>   | 2005 | US/Caucasian         | Healthy control  | Applied biosystems | 151   | 189     | 109                   | 35  | 7   | 124     | 57    | 8     | 0.66                         | TLE       | Adult | 0.19 |
| Bragatti et al <sup>22</sup> | 2010 | Brazil/Caucasian     | Healthy control  | PCR-RFLP           | 101   | 104     | 73                    | 28  | 0   | 74      | 30    | 0     | 0.09                         | TLE       | Adult | 0.14 |
| Chen et al <sup>23</sup>     | 2011 | China/Asian          | Hospital control | PCR-RFLP           | 128   | 132     | 33                    | 66  | 29  | 33      | 70    | 29    | 0.48                         | Epilepsy  | Child | 0.48 |
| Tondo et al <sup>29</sup>    | 2011 | Spain/Caucasian      | NA               | Applied biosystems | 10    | 56      | 8                     | 1   | 1   | 36      | 17    | 3     | 0.60                         | Epilepsy  | Mixed | 0.21 |
| Fu et al <sup>24</sup>       | 2014 | China/Asian          | Hospital control | Direct sequencing  | 40    | 40      | 13                    | 18  | 9   | 10      | 17    | 13    | 0.36                         | TLE+other | Child | 0.54 |
| Gkampeta et al <sup>25</sup> | 2014 | Greece/Caucasian     | Healthy control  | PCR-RFLP           | 60    | 60      | 38                    | 19  | 3   | 32      | 24    | 4     | 0.86                         | Epilepsy  | Child | 0.27 |
| Sha'ari et al <sup>26</sup>  | 2016 | Malaysia+China/Asian | Healthy control  | Illumina HumanHap  | 1,630 | 4,401   | 500                   | 780 | 350 | 1,201   | 2,166 | 1,034 | 0.34                         | Epilepsy  | Adult | 0.48 |
| Shen et al <sup>27</sup>     | 2016 | China/Asian          | Hospital control | Taqman             | 499   | 1,181   | 158                   | 244 | 97  | 331     | 551   | 299   | 0.02                         | TLE       | Adult | 0.49 |

<sup>a</sup>Note: <sup>a</sup>HWE in control.

**Abbreviations:** AD, age distribution; HWE, Hardy-Weinberg equilibrium; MAF, minor allele frequency in control group; NA, not available; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; TLE, temporal lobe epilepsy.

assess the between-study heterogeneity. The fixed-effect model (the Mantel-Haenszel method) was used when the  $I^2$  index was less than 40%; otherwise, a random effects model (the DerSimonian and Laird method) was applied. Furthermore, cumulative analyses and sensitivity analyses were conducted to examine the trends and the stability of the results. Publication bias was conducted with Begg's funnel plot and Egger's test. Statistical analyses were performed with STATA version 14.0 (StataCorp LP, College Station, TX, USA).  $P<0.05$  was statistically significant.

## Results

### Study characteristics

In general, 211 studies were identified by a systematic literature search. Figure 1 presents the selection process of the studies. After screening the titles, abstracts, and full articles, 201 articles were excluded. A total of 10 studies involving 2,941 patients and 6,571 controls were included in this meta-analysis.<sup>19,21–29</sup> Among these studies, five studies focused on adult patients,<sup>19,21,22,26,27</sup> four studies focused on pediatric patients,<sup>23–25,28</sup> and one study focused on mixed patients.<sup>29</sup> Six studies researched Asian populations<sup>9,23,24,26–28</sup> and four researched Caucasian populations.<sup>21,22,25,29</sup> Five studies focused on the subtype of temporal lobe epilepsy.<sup>19,21,22,24,27</sup> In the genotyping method subgroup, four studies used polymerase chain reaction-restriction fragment length polymorphism,<sup>19,22,23,25,28</sup> two studies used applied biosystems,<sup>21,29</sup> one study adopted direct sequencing,<sup>24</sup> one study used Illumina HumanHap,<sup>26</sup> and the last study applied the Taqman method.<sup>27</sup> In terms of the genotype distributions in control groups, only one study deviated from HWE.<sup>27</sup> All included characteristics of each study are summarized in Table 1.

### Meta-analysis

Meta-analysis was conducted for the combined studies, and the results demonstrated some significant protective effects between rs6265 G>A polymorphism and epilepsy susceptibility in all five genotype models (A vs G: OR=0.88, 95% CI=0.83–0.94,  $P<0.01$ ,  $I^2=0\%$ ; GA vs GG: OR=0.88, 95% CI=0.79–0.97,  $P=0.01$ ,  $I^2=0\%$ ; AA vs GG: OR=0.79, 95% CI=0.70–0.90,  $P<0.01$ ,  $I^2=0\%$ ; GA+AA vs GG: OR=0.85, 95% CI=0.77–0.94,  $P<0.01$ ,  $I^2=0\%$ , Figure 2; AA vs GG+GA: OR=0.85, 95% CI=0.76–0.95,  $P=0.01$ ,  $I^2=0\%$ ) (Figure S1 shows other models). Subsequently, analysis excluding the study by Shen et al, which deviated from HWE, also indicated decreased risks in the five genotype models (Table 2).

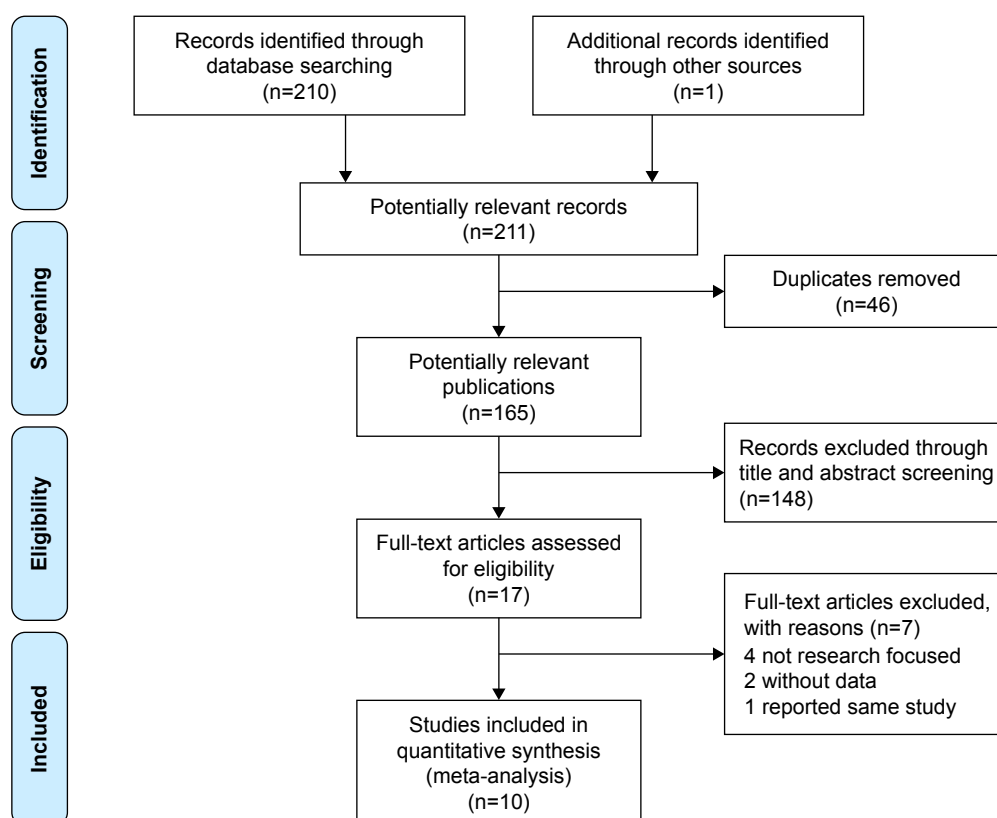


Figure 1 Flow diagram of the study selection process.

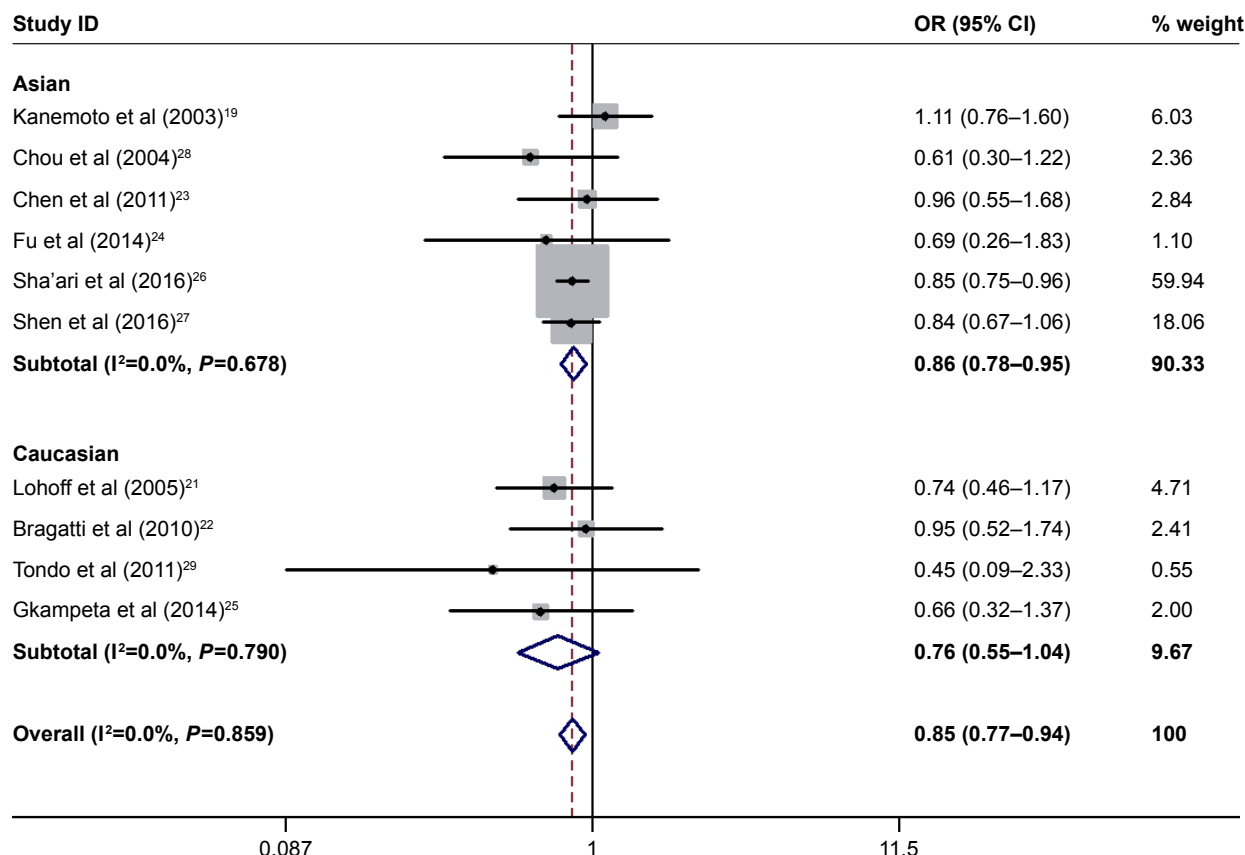


Figure 2 OR and 95% CIs of the associations between *BDNF* rs6265 G>A polymorphism and epilepsy susceptibility in GA+AA vs GG model.

Table 2 Summary ORs and 95% CIs of BDNF rs6265 G&gt;A polymorphism and epilepsy risk

| rs6265 G>A     | N* | A vs G |           |         |                    | GA vs GG |           |         |                    | AA vs GG |            |         |                    | GA+AA vs GG |           |         |                    | AA vs GG+GA |            |         |                    |
|----------------|----|--------|-----------|---------|--------------------|----------|-----------|---------|--------------------|----------|------------|---------|--------------------|-------------|-----------|---------|--------------------|-------------|------------|---------|--------------------|
|                |    | OR     | 95% CI    | P-value | I <sup>2</sup> (%) | OR       | 95% CI    | P-value | I <sup>2</sup> (%) | OR       | 95% CI     | P-value | I <sup>2</sup> (%) | OR          | 95% CI    | P-value | I <sup>2</sup> (%) | OR          | 95% CI     | P-value | I <sup>2</sup> (%) |
| Total          | 10 | 0.88   | 0.83–0.94 | <0.01   | 0                  | 0.88     | 0.79–0.97 | 0.01    | 0                  | 0.79     | 0.70–0.90  | <0.01   | 0                  | 0.85        | 0.77–0.94 | <0.01   | 0                  | 0.85        | 0.76–0.95  | 0.01    | 0                  |
| HWE-yes        | 9  | 0.90   | 0.84–0.96 | 0.01    | 0                  | 0.87     | 0.77–0.97 | 0.01    | 0                  | 0.82     | 0.71–0.95  | 0.01    | 0                  | 0.85        | 0.77–0.95 | <0.01   | 0                  | 0.89        | 0.79–1.01  | 0.07    | 0                  |
| Ethnicity      |    |        |           |         |                    |          |           |         |                    |          |            |         |                    |             |           |         |                    |             |            |         |                    |
| Asian          | 6  | 0.89   | 0.83–0.95 | <0.01   | 0                  | 0.89     | 0.80–0.99 | 0.04    | 0                  | 0.79     | 0.69–0.90  | <0.01   | 0                  | 0.86        | 0.78–0.95 | <0.01   | 0                  | 0.85        | 0.76–0.95  | 0.01    | 0                  |
| Caucasian      | 4  | 0.82   | 0.62–1.08 | 0.15    | 0                  | 0.73     | 0.52–1.03 | 0.07    | 0                  | 0.92     | 0.40–2.08  | 0.84    | 0                  | 0.76        | 0.55–1.04 | 0.09    | 0                  | 1.04        | 0.46–2.34  | 0.92    | 0                  |
| Design         |    |        |           |         |                    |          |           |         |                    |          |            |         |                    |             |           |         |                    |             |            |         |                    |
| Hospital base  | 4  | 0.88   | 0.79–0.99 | 0.04    | 13.2               | 0.97     | 0.80–1.17 | 0.75    | 0                  | 0.77     | 0.61–0.98  | 0.03    | 0                  | 0.90        | 0.76–1.08 | 0.27    | 0                  | 0.79        | 0.64–0.97  | 0.02    | 0                  |
| Healthy base** | 5  | 0.88   | 0.82–0.95 | <0.01   | 0                  | 0.84     | 0.75–0.95 | 0.01    | 0                  | 0.80     | 0.69–0.94  | 0.01    | 0                  | 0.83        | 0.74–0.93 | <0.01   | 0                  | 0.88        | 0.77–1.01  | 0.06    | 0                  |
| NA             | 1  | 0.68   | 0.18–2.53 | 0.57    | NA                 | 0.26     | 0.03–2.29 | 0.23    | NA                 | 1.50     | 0.14–16.36 | 0.74    | NA                 | 0.45        | 0.09–2.33 | 0.34    | NA                 | 1.96        | 0.18–21.02 | 0.58    | NA                 |
| Subtype        |    |        |           |         |                    |          |           |         |                    |          |            |         |                    |             |           |         |                    |             |            |         |                    |
| TLE            | 5  | 0.88   | 0.78–0.99 | 0.04    | 39.3               | 0.89     | 0.74–1.07 | 0.23    | 0                  | 0.80     | 0.62–1.02  | 0.07    | 0                  | 0.86        | 0.72–1.03 | 0.10    | 0                  | 0.73        | 0.57–1.52  | 0.77    | 60.8               |
| AD             |    |        |           |         |                    |          |           |         |                    |          |            |         |                    |             |           |         |                    |             |            |         |                    |
| Adult          | 5  | 0.89   | 0.83–0.95 | <0.01   | 0                  | 0.89     | 0.80–0.99 | 0.03    | 0                  | 0.80     | 0.70–0.92  | <0.01   | 0                  | 0.86        | 0.78–0.95 | <0.01   | 0                  | 0.86        | 0.76–0.96  | 0.01    | 0                  |
| Children       | 4  | 0.82   | 0.66–1.03 | 0.09    | 0                  | 0.78     | 0.54–1.13 | 0.20    | 0                  | 0.71     | 0.45–1.12  | 0.14    | 0                  | 0.75        | 0.53–1.07 | 0.11    | 0                  | 0.81        | 0.55–1.18  | 0.28    | 0                  |
| Mixed          | 1  | 0.68   | 0.18–2.53 | 0.57    | NA                 | 0.26     | 0.03–2.29 | 0.23    | NA                 | 1.50     | 0.14–16.36 | 0.74    | NA                 | 0.45        | 0.09–2.33 | 0.34    | NA                 | 1.96        | 0.18–21.02 | 0.58    | NA                 |
| Type           |    |        |           |         |                    |          |           |         |                    |          |            |         |                    |             |           |         |                    |             |            |         |                    |
| PCR-RFLP       | 5  | 0.94   | 0.79–1.10 | 0.43    | 0                  | 0.94     | 0.73–1.21 | 0.63    | 0                  | 0.88     | 0.61–1.26  | 0.48    | 0                  | 0.92        | 0.73–1.17 | 0.52    | 0                  | 0.91        | 0.67–1.23  | 0.53    | 0                  |
| Other          | 5  | 0.87   | 0.82–0.94 | <0.01   | 0                  | 0.86     | 0.77–0.97 | 0.01    | 0                  | 0.78     | 0.68–0.90  | <0.01   | 0                  | 0.84        | 0.75–0.93 | <0.01   | 0                  | 0.85        | 0.75–0.95  | 0.01    | 0                  |

**Notes:** Statistically significant values are shown in bold. *I*<sup>2</sup> for heterogeneity test. \*Numbers of comparisons. \*\*Healthy base refers to control groups that consist of healthy persons who may be from the hospital or from the general population.

**Abbreviations:** AD, age distribution; HWE, Hardy-Weinberg equilibrium; NA, not available; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; TLE, temporal lobe epilepsy.

Furthermore, the subgroup analyses were stratified by ethnicity, control design, disease type, age distribution, and genotyping method. All these findings presented consistent effects with the overall analysis results, such as in Asians (A vs G: OR=0.89, 95% CI=0.83–0.95,  $P<0.01$ ,  $I^2=0\%$ ; GA vs GG: OR=0.89, 95% CI=0.80–0.99,  $P=0.04$ ,  $I^2=0\%$ ; AA vs GG: OR=0.79, 95% CI=0.69–0.90,  $P<0.01$ ,  $I^2=0\%$ ; GA+AA vs GG: OR=0.86, 95% CI=0.78–0.95,  $P<0.01$ ,  $I^2=0\%$ ; AA vs GG+GA: OR=0.85, 95% CI=0.76–0.95,  $P=0.01$ ,  $I^2=0\%$ ) and adults (A vs G: OR=0.89, 95% CI=0.83–0.95,  $P<0.01$ ,  $I^2=0\%$ ; GA vs GG: OR=0.89, 95% CI=0.80–0.99,  $P=0.03$ ,  $I^2=0\%$ ; AA vs GG: OR=0.80, 95% CI=0.70–0.92,  $P<0.01$ ,  $I^2=0\%$ ; GA+AA vs GG: OR=0.86, 95% CI=0.78–0.95,  $P<0.01$ ,  $I^2=0\%$ ; AA vs GG+GA: OR=0.86, 95% CI=0.76–0.96,  $P=0.01$ ,  $I^2=0\%$ ) (Table 2).

Sensitivity analyses were performed by removing each study one-by-one according to the publication date, and the results did not show significant changes (Figure 3 for GA+AA vs GG model and Figure S2 for other models). Cumulative analysis was also performed and presented an apparent protective effect in 2016 from the report by Sha'ari et al (Figure 4 for GA+AA vs GG model and Figure S3 for other models).

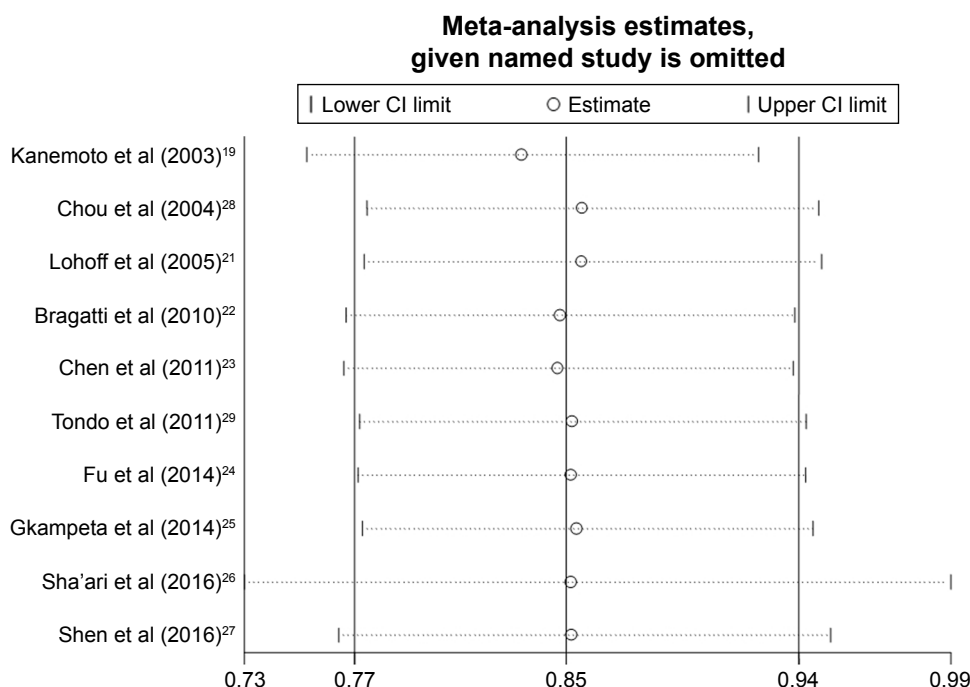
Publication bias was evaluated with Begg's tests, and no significant asymmetry was found (Figure 5 for GA+AA vs GG model and Figure S4 for other models). The Egger's test

also confirmed these results (A vs G,  $P=0.39$ ; GA vs GG:  $P=0.33$ ; AA vs GG,  $P=1.00$ ; GA+AA vs GG,  $P=0.39$ ; AA vs GG+GA,  $P=0.99$ ).

## Discussion

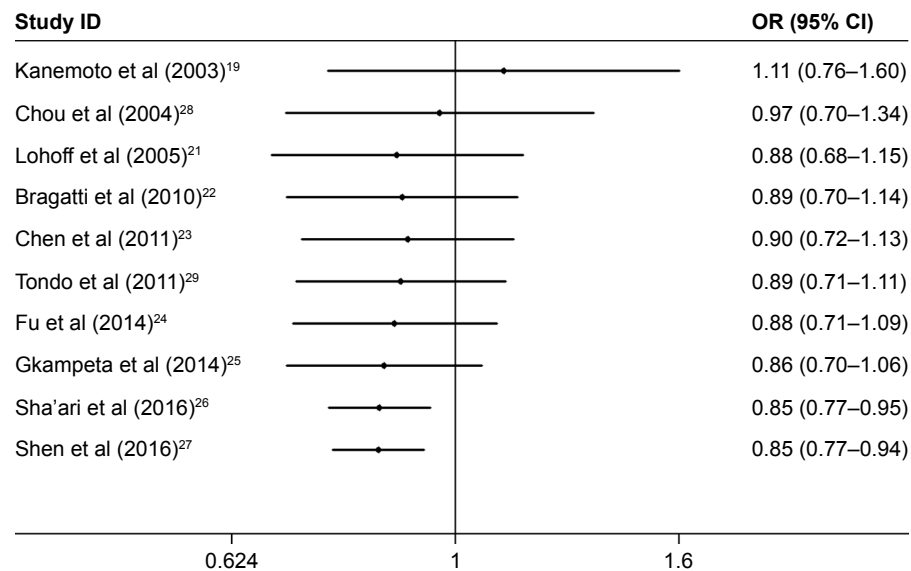
Epilepsy is one of the most common neurological conditions triggered by the disruption of the abnormal electrochemical activities of the brain's neurological system, thereby resulting in a seizure. Epilepsy affects approximately 0.5% to 1% of the general population. Epilepsy can be controlled, but not cured, and more than 30% of patients have a poor response to medical treatment.<sup>30</sup>

Goldberg and Coulter suggested that the etiology of epilepsy in most patients is still unclear, especially for idiopathic epilepsies, which, without any specific cause, contributed to more than 70% of seizure symptoms.<sup>31</sup> However, Scheffer et al postulated that idiopathic epilepsies, nowadays, are known to be of genetic origin, particularly those with early onset – in the first or second decade of life. On the other hand, many symptomatic epilepsies are now classified as structural, metabolic, genetic, post-inflammatory, post-traumatic, or a combination of these etiologies.<sup>32</sup> BDNF is one of the crucial nerve growth factors in the family of neurotrophins and plays an important role in the maintenance, regulation, and differentiation of peripheral and central nervous system neurons.<sup>33</sup> A large amount of research has indicated that abnormal



**Figure 3** Sensitivity analysis by removing one study at a time to reflect the influence of the individual dataset on the pooled ORs in GA+AA vs GG model of *BDNF* rs6265 G>A polymorphism.



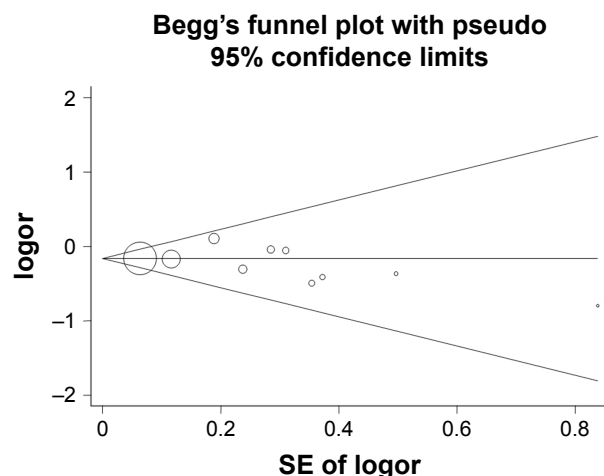


**Figure 4** Cumulative meta-analyses according to publication year in GA+AA vs GG model of *BDNF* rs6265 G>A polymorphism.

expression of BDNF plays a critical role in the pathological process of epilepsy development. Martínez-Levy et al and de Souza Bernardino et al found an increased expression of BDNF transcription in patients with epilepsy, which also supported the idea that the high expression of BDNF in some brain areas may contribute to limbic seizures.<sup>34,35</sup> Moreover, transgenic mice with high expression of BDNF also indicated an increased seizure risk combined with a hippocampal hyperexcitability.<sup>13</sup> This cumulative evidence prompted the idea that the abnormalities in the *BDNF* gene may be responsible for epileptogenesis.

The human *BDNF* gene is located at the short arm of chromosome 11p13.<sup>36</sup> Today, several SNP loci of the *BDNF*

gene have been found, and the rs6265 variant is the most common site. This polymorphism substitutes a guanine to adenine (G>A) change at the nucleotide 196 site and results in a Val to Met substitution at amino acid residue 66 of the pro-BDNF protein.<sup>37</sup> Bhang et al found that the Met allele of the *BDNF* val66met polymorphism contributed to a significantly lower serum level of BDNF.<sup>38</sup> In addition, some studies indicated that the variant-type *BDNF* (*BDNF* Met) would create negative effects to alter the trafficking paths with two important aspects: 1) decreased variant *BDNF* distributed into neuronal dendrites and targeted to secretory granules, 2) contributed to the impairment of regulated secretion of BDNF.<sup>39,40</sup> Furthermore, other researchers also found that this change reduces the activity-dependent secretion of BDNF and influences cortical processing,<sup>40–42</sup> such as low BDNF expression, and protects against seizures with the Met allele of this polymorphism.<sup>40,43</sup> In 2003, Kanemoto et al conducted the first case–control study to explore the association between the *BDNF* rs6265 G>A polymorphism and epilepsy susceptibility in the Japanese population, and the results indicated no statistically significant differences between the epilepsy patients and control groups.<sup>19</sup> Similarly, Lohoff et al and Bragatti et al did not find any significant association between *BDNF* rs6265 G>A polymorphism and epilepsy risk.<sup>21,22</sup> Since then, a series of studies have been published, but the results were controversial and inconsistent. Sha'ari et al performed a case–control study on an East Asian population and found that the population had a higher risk of developing epilepsy compared with G carriers in all subjects (dominant model, OR=1.18, 95% CI=1.04–1.33).<sup>26</sup>



**Figure 5** Funnel plot analysis to detect publication bias for GA+AA vs GG model of *BDNF* rs6265 G>A polymorphism.

**Note:** Circles represent the number of the studies.

Conversely, Shen et al suggested that the A allele might play a protective role against epilepsy risk.<sup>27</sup>

These inconsistent results create confusion. How can we conduct a more precise assessment of the association between *BDNF* rs6265 G>A polymorphism and epilepsy risk? Few studies and small sample size may be the most important factors that contributed to the confusing results. Meta-analysis is a statistical method that combines the results of multiple studies focused on one topic. Therefore, we conducted this meta-analysis with published studies involving a total of 9,512 subjects and found that the A allele mutation of *BDNF* rs6265 G>A polymorphism plays an important protective role against epilepsy development, especially in the Asian population. This result might be due to the greater number of studies conducted in Asian populations with large sample sizes, thereby providing increased statistical efficiency. Moreover, the subgroup analyses based on control design, disease type, age distribution, and genotyping method also indicated some protective effect against epilepsy. Interestingly, the mechanism of *BDNF* rs6265 G>A polymorphism was more effective in the adult group compared with the pediatric group. It was supposed that the accumulative protective effect of *BDNF* rs6265 G>A mutation would present gradually along with aging.

To the best of our knowledge, this is the first meta-analysis to assess the association between *BDNF* rs6265 G>A polymorphism and epilepsy susceptibility.

## Limitations

Some limitations need to be addressed in the present analysis. First, although we conducted a systematic review and collected eight published studies, the sample size was still small, which could have influenced the results, thereby resulting in some deviations from the truth. Second, only one polymorphism locus (*BDNF* rs6265 G>A) was assessed in our analysis. Some influencing factors, such as brain trauma; cigarette smoking, alcohol, and drug use during pregnancy; environmental deterioration; and other gene mutations, could not be comprehensively evaluated. Third, all the included studies were conducted in Caucasian and Asian population. Ethnicity bias may have affected the results and might not be applicable to all people. Despite these flaws, the findings further help us understand the potential associations between *BDNF* rs6265 G>A polymorphism and epilepsy susceptibility. Furthermore, some positive aspects have been addressed: 1) no heterogeneity exists between the included studies, 2) the results of sensitivity analysis and cumulative analysis are all

fairly consistent, and 3) Egger's test and Begg's funnel plots did not present any significant publication bias.

## Conclusion

In summary, this meta-analysis indicated that the *BDNF* rs6265 G>A polymorphism is associated with epilepsy development, especially in Asian population. More studies with large sample sizes and in-depth research on the mechanism are needed to explore the role of *BDNF* rs6265 G>A polymorphism.

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## Disclosure

The authors report no conflicts of interest in this work.

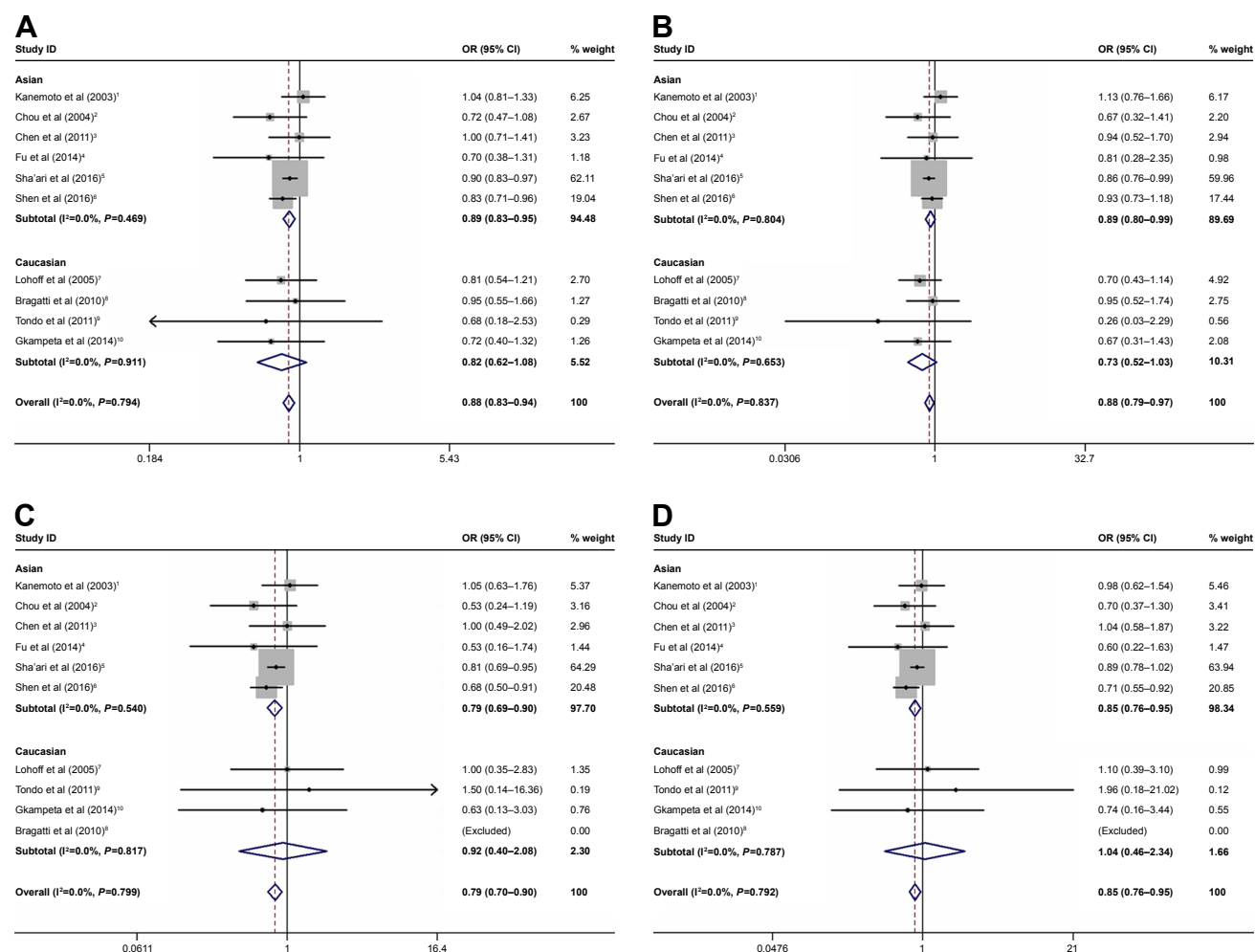
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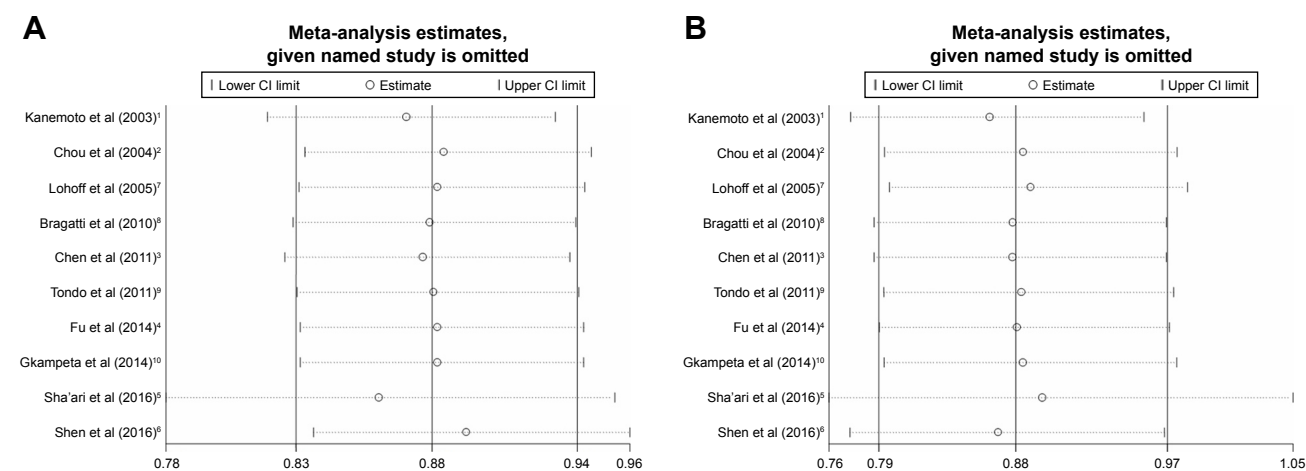


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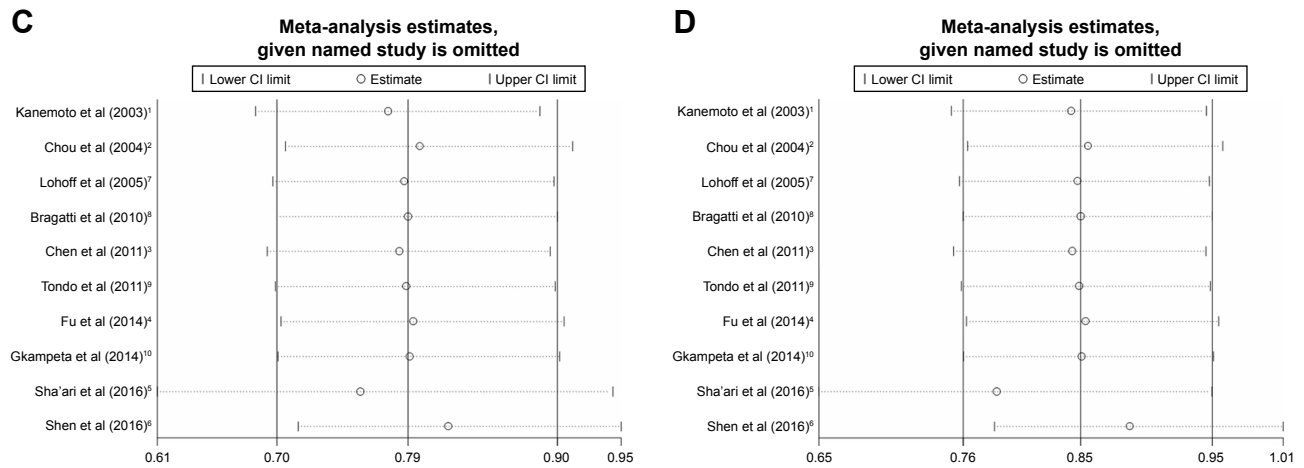
## Supplementary materials



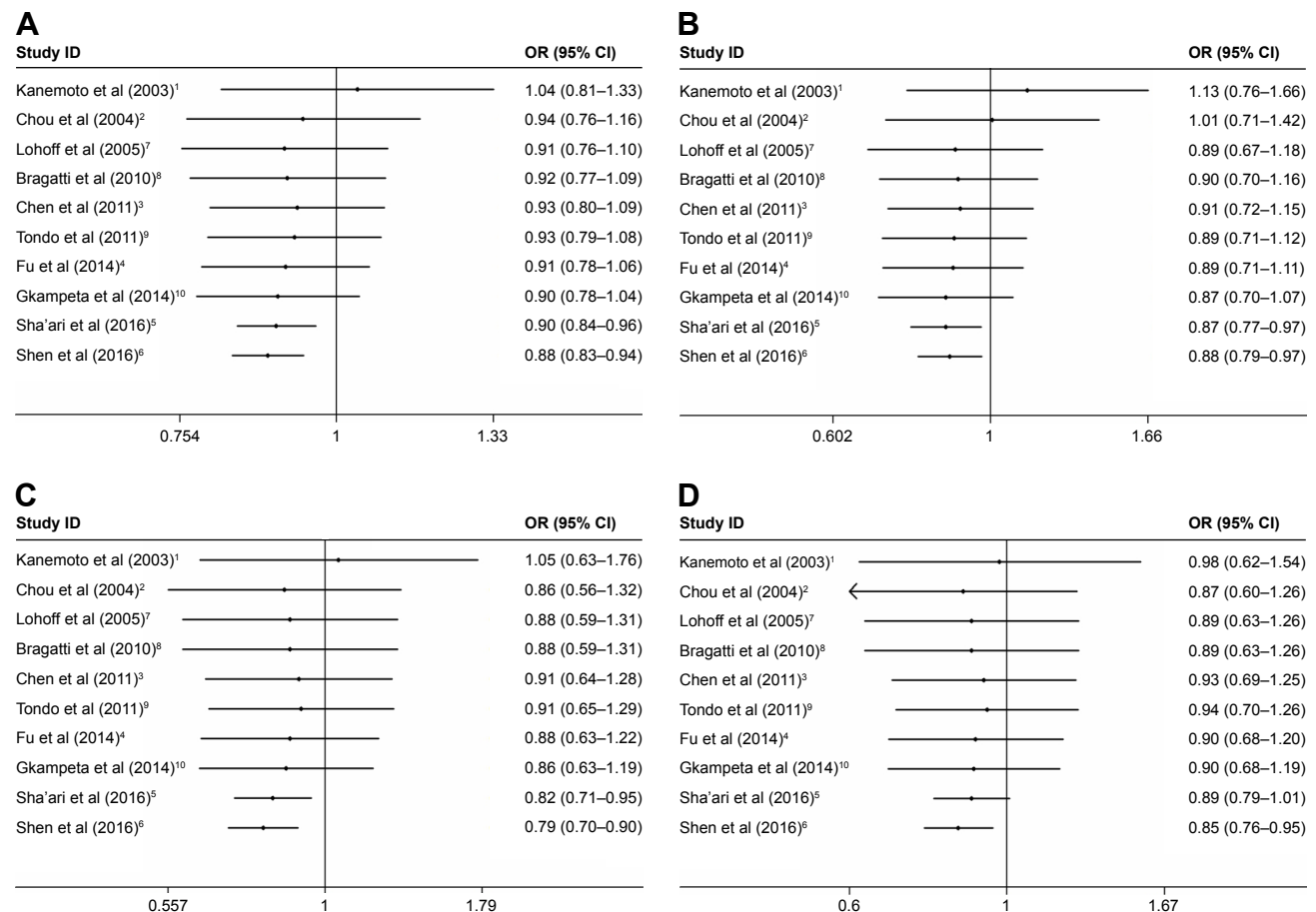
**Figure S1** OR and 95% CIs of the associations between BDNF rs6265 G>A polymorphism and epilepsy susceptibility (**A** for A vs G model; **B** for GA vs GG model; **C** for AA vs GG model; and **D** for AA vs GG+GA model).



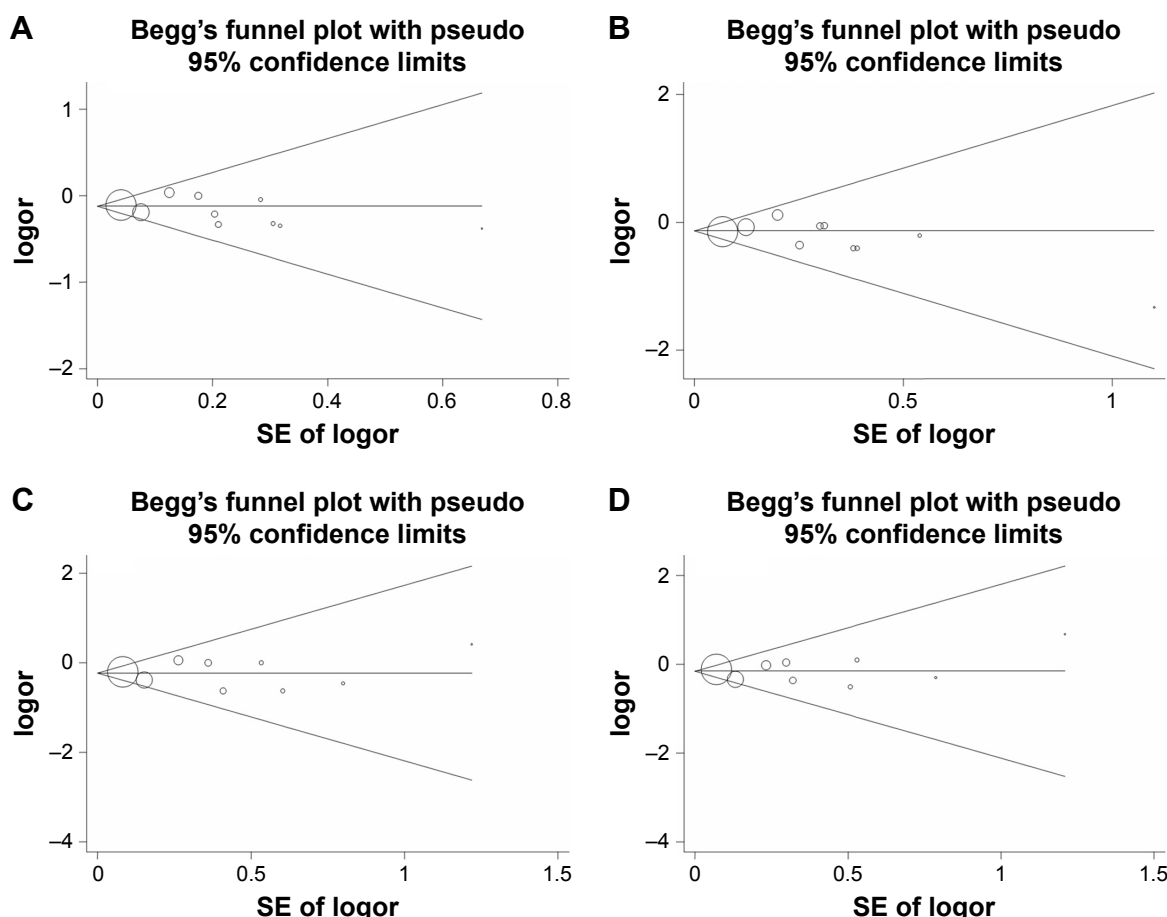
**Figure S2** (Continued)



**Figure S2** Sensitivity analysis through deleting each study to reflect the influence of the individual dataset to the pooled ORs in BDNF rs6265 G>A polymorphism and epilepsy susceptibility (**A** for A vs G model; **B** for GA vs GG model; **C** for AA vs GG model; and **D** for AA vs GG+GA model).



**Figure S3** Cumulative meta-analyses according to publication year in BDNF rs6265 G>A polymorphism and epilepsy susceptibility (**A** for A vs G model; **B** for GA vs GG model; **C** for AA vs GG model; and **D** for AA vs GG+GA model).



**Figure S4** Funnel plot analysis to detect publication bias in BDNF rs6265 G>A polymorphism and epilepsy susceptibility (**A** for A vs G model; **B** for GA vs GG model; **C** for AA vs GG model; and **D** for AA vs GG+GA model).

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